Separation of Azoxybenzenes and some derivatives by High Performance Liquid Chromatography.

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Key words: HPLC, Azoxybenzene, Gas chromatography, photoreaction.

Abstract

A high performance liquid chromatographic method was used for the Separation and determination of a mixture of Azoxybenzene (diphenyldiazine-1-oxide) together with three of its derivatives; i.e. 4,'4-dinitroazoxy benzene; 4, '4-dichloroazoxybenzene and 4-chloro-'4-methoxyazoxybenzene.Chromatography was performed at 50C° using a mixture of methanol-water as a mobile phase. The percentage of methanol in this mixture was between (80-90)%. The precision was very high since the error in this study was less than (0.3%) because the relative error of percentage Erel% is between (0.28-0.65) and the detection limit (DL) in this method was equal to (0.2) ng.

Introduction

The qualitative and quantitative determination of disubstituted azoxy benzene is most important for the in-process and final product quality control.

Methods previously reported for the problem of the azoxybenzene analysis require thin-layer chromatography[1,2].Gas chromatography[3.4] is not suitable for precise analysis because of the thermal instability of the azoxybenzene.

In this report we describe a reliable method for the separation and determination of disubstituted azoxybenzene by isocratic phase performance liquid chromatography.

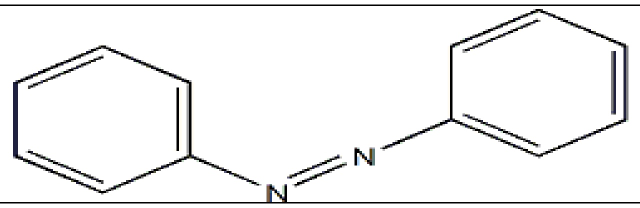
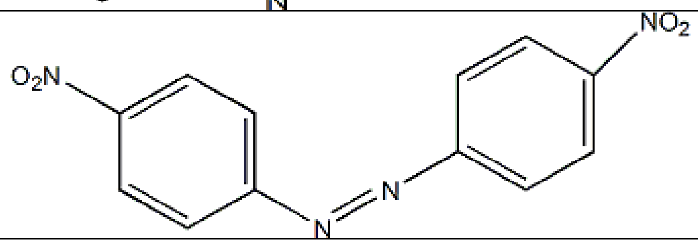
Gas chromatography/mass spectrometry (GC/MS) is the most widely used general technique for the identification of volatile organic compounds in complex mixtures[5,6]. In many cases compounds in such mixtures are inefficiently separated by GC, but not always. Liquid-Liquid partitioning methods have often been used to fractionate complex mixtures. Novotny et al.[7,8,9,10] developed a liquid-liquid partitioning method to isolate polynuclear aromatic hydrocarbons in complex mixtures.

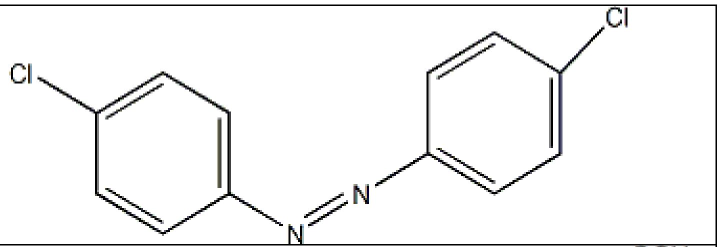
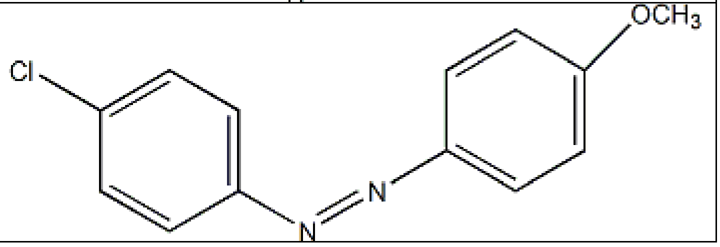
It is usually accepted that particles of a (5-10) µm in size have to be packed by slurry techniques. Solvent systems with suitable equal density or viscosity properties are used to keep the particles in a reasonably suspended state during the packing procedures[1,2]. In literature an alternative method was proposed where silica gel and alumina particles of (3-6) µm in size were packed upward with methanol as a slurry solvent. The column dimensions varied between (10-100) cm. In length and $\geq$5 mm i.d. was used because the few experimental data published, it was difficult to make a comparison with the method presently discussed [3].

Experimental

Reagents:

Azoxybenzene together with the following three disubstituted derivatives were studied:

1-	Azoxybenzene (C_6H_5)-N=N-(C_6H_5) Gfwt :214.212 Purity :99.82% Aldrich chemical co.	
2-	4,4-dinitroazoxybenzene $NO_2(C_6H_5)$-N=N-$(C_6H_5)NO_2$ Gfwt :304.510 Purity :99.92% Aldrich chemical co.	

3-	4,4-dichloroazoxybenzene Cl(C_6H_5)-N=N-(C_6H_5) Cl Gfwt :299.324 Purity :99.94% Aldrich chemical co.	
4-	4-chloro-4-methoxyazoxybenzene Cl(C_6H_5)-N=N-(C_6H_5)-OCH_3 Gfwt :294.425 Purity :99.9% Aldrich chemical co.	

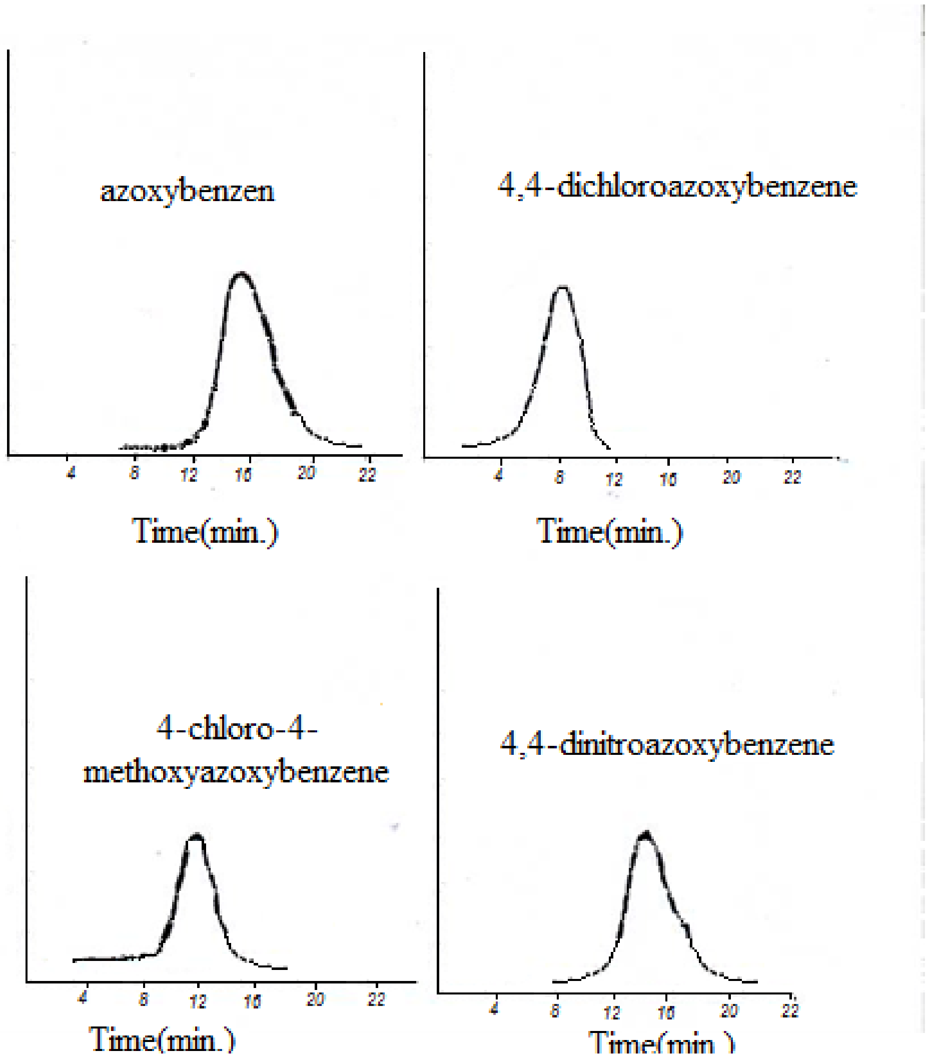


Fig (1) HPLC chromatograms of azoxybenzene and the three derivatives,a:azoxybenzene b: 4,4-dichloroazoxybenzene, c:4-chloro-4-methoxyazoxybenzene d: 4,4-dinitroazoxybenzene, mobile phase (80%metanol)

Result and Discussion

The high performance liquid chromatography showed in figs (2, 3, 4) the chromatogram of azoxybenzene and fig(5,6,7)and in fig (8) that of 4,'4-disubstituted azoxybenzenes obtained with several methanol mobile phases containing from (80-90% v/v methanol water solution).

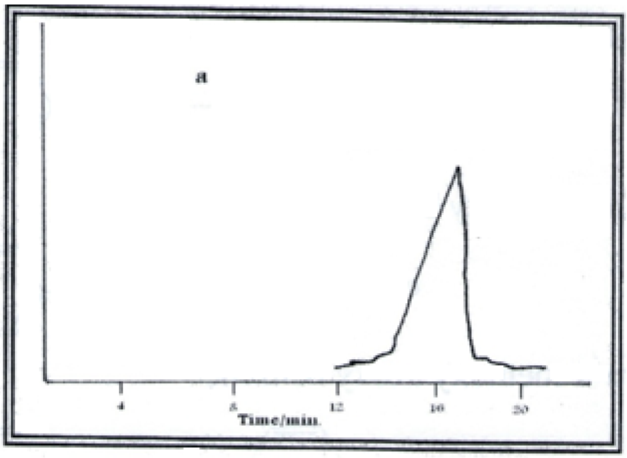


Fig (2): HPLC chromatograms of azoxybenzenes separated with mobile phase (80% v/v Methanol).

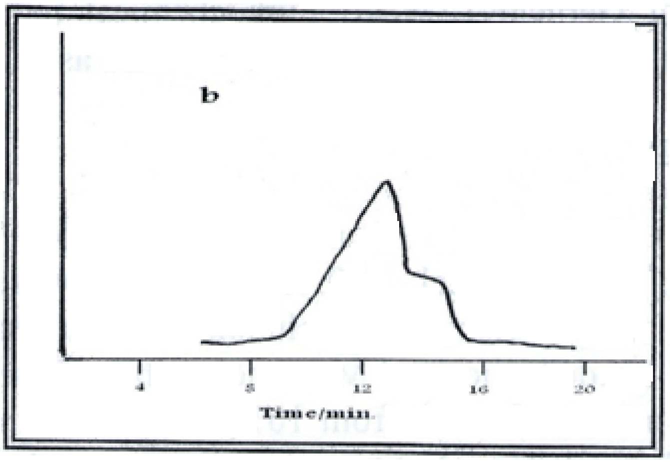


Fig (3): HPLC chromatograms of azoxybenzenes separated with mobile phase (85% v/v Methanol).

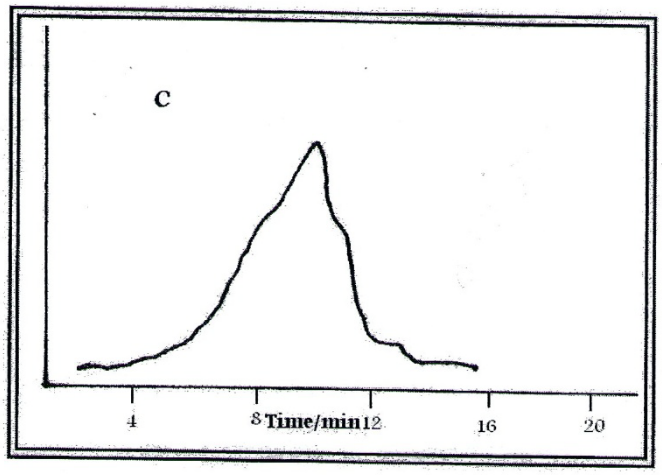
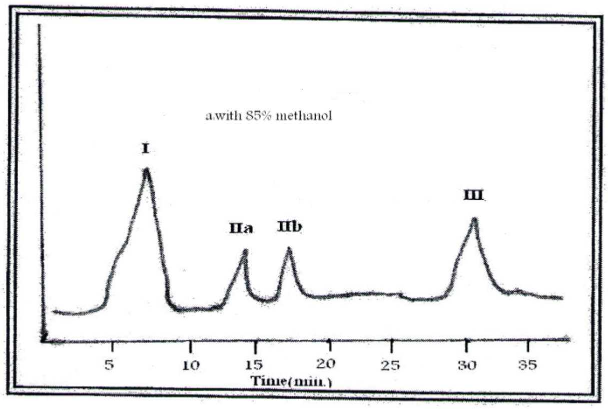


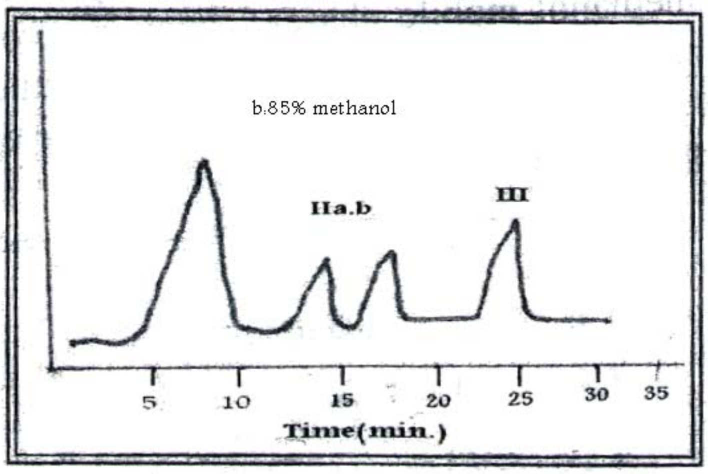
Fig (4): HPLC chromatograms of azoxybenzenes separated with mobile phase (90% Methano1 v/v).

When the % distilled water polarity increases in the mobile phase the retention time of azoxybenzene in the column increases and its peak becomes sharper[11].

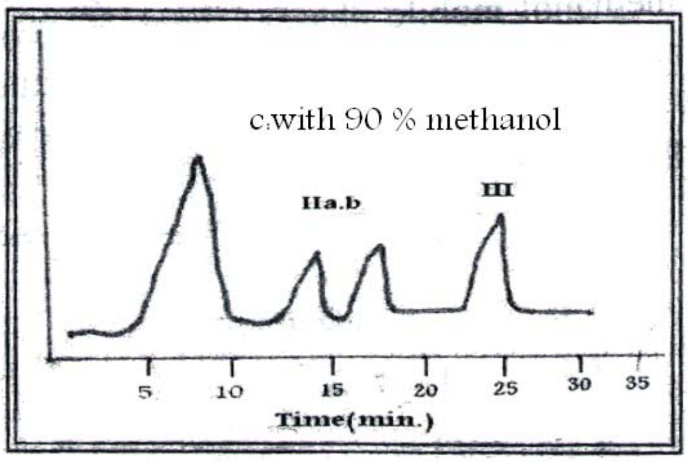
Fig(5,6 and 7) showed the high performance liquid chromatographic chromatograms of 4,4-disubstituted azoxybenzene obtained with several methanol mobile phases containing from(10 to 20% v/v of water).The effect of an nitro group substituted onto n carbon atoms on the capacity factor is approximately different as that for halide substituent.



Fig(5):Chromatograms of I: 4,`4-disubstituted azoxybenzenes II:4,'4-dichloro azoxybenzene III: azoxybenzene,IV: 4,4-dinitroazoxybenzene,mobile phase (80%v/v methanol).



Fig(6): Chromatograms of I: 4,`4-disubstituted azoxybenzenes II:4,'4-dichloro azoxybenzene III: azoxybenzene,IV: 4,4-dinitroazoxybenzene,mobile phase (85% v/v methanol solution).



Fig(7): Chromatograms of I: 4,`4-disubstituted azoxybenzenes II:4,'4-dichloro azoxybenzene III: azoxybenzene,IV: 4,4-dinitroazoxybenzene, mobile phase (90% v/v methanol).

Fig (8) shows the unsymmetrical substituted azoxybenzene II (4-chloro-'4-methoxyazoxybenzene). Yields two adjacent peaks with methanol mobile phases having higher water content, these two peaks are attributable to geometrical isomers. In order to prove this, infrared analysis of the eluted peaks was carried out.

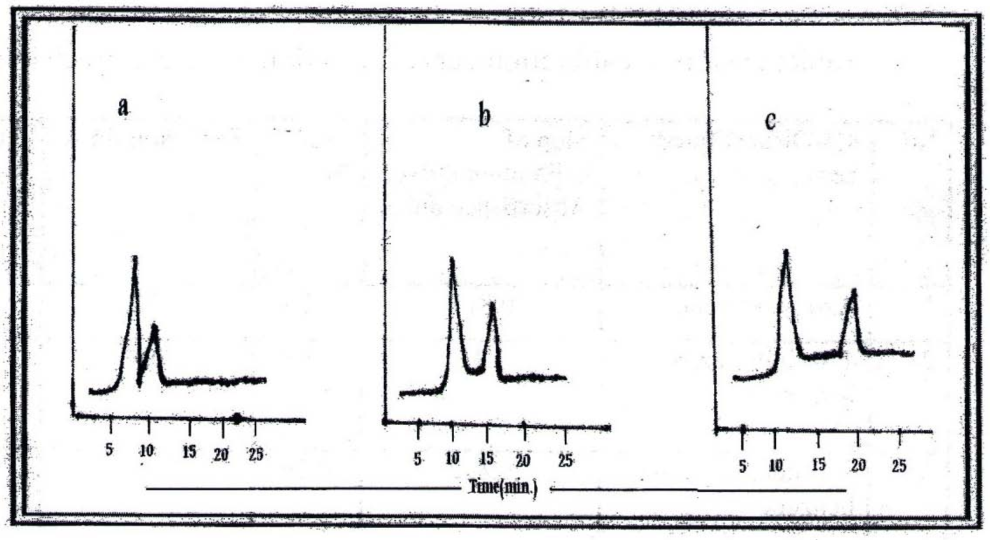


Fig (8): Chromatograms of 4-chloro-'4-methoxy azoxybenzene show the effect of methanol percentage which a: 90% v/v methanol.b:85%v/v,c: 80% v/v with a flow rate of 1.2cm^3/min at50C°.

The two eluted peaks for 4-chloro-'4-methoxy azoxy benzene were fraction-collected with a methanol mobile phase containing 80% v/v of methanol differential refract meter detector was used because the photoreaction of azoxy benzene derivative might be induced by ATV irradiation[12]. The same,I.R. spectra were obtained for the first peak and the second one.The infrared spectra suggested that these compounds were azoxy benzene derivatives in which the two benzene rings were paradisubstituted and that one of their substituents was a methoxy group.From the results of the infrared described above, it is concluded that the two peaks for the (IV) are attributable to the geometrical isomers of IV.

Table(2) show slope of calibration curve, Relative Standard and Deviation of slope, Detection limit and Precision for 2x10^{-3} M (4,`4 -disubstituted azoxy benzenes (Mobile phase 85% of methanol in water, flow rate 1-2 ml min^{-1} at 50 C°,amount of azoxy benzenes injected 0.12ml.

Table (1) the concentration take and concentration found of the azoxybenzene and the three derivatives.

No	Compound	Conc. taken Mole/L	Conc. Found Mole/L	Er%	Avarge error percent
1-	azoxybenzen	$2x10^{-3}$ $1x10^{-3}$ $0.7x10^{-3}$ $0.5x10^{-3}$	$1.995x10^{-3}$ $9.7x\ 10^{-4}$ $6.919\ x10^{-4}$ $4.986\ x10^{-4}$	-0.25 -0.30 -0.28 -0.28	$28x10^{-2}$
2-	4,4-dichloroazoxybenzen	$2x10^{-3}$ $1x10^{-3}$ $0.7x10^{-3}$ $0.5x10^{-3}$	$1.996x10^{-3}$ $9.96x\ 10^{-4}$ $6.998\ x10^{-4}$ $4.998\ x10^{-4}$	-0.21 -0.19 -0.20 -0.20	$2x10^{-1}$
3-	4-chloro4-methoxyazoxybenzen	$2x10^{-3}$ $1x10^{-3}$ $0.7x10^{-3}$ $0.5x10^{-3}$	$1.996x10^{-3}$ $9.98x\ 10^{-4}$ $6.98\ x10^{-4}$ $4.999\ x10^{-4}$	-0.19 -0.20 -0.195 -0.185	$1.9x10^{-1}$
4-	4,4-dinitroazoxybenzen	$2x10^{-3}$ $1x10^{-3}$ $0.7x10^{-3}$ $0.5x10^{-3}$	$1.994x10^{-3}$ $9.997x\ 10^{-4}$ $6.997\ x10^{-4}$ $4.998\ x10^{-4}$	-0.3 -0.29 -0.31 -0.30	$3x10^{-1}$

Table(2):slop of calibration curve, detection limit and precision.

No	4,4- Disubstituted azoxy benzene	Slop of calibration curve.	Erel.%	Detection limit PPm	Precision%
1	azoxy benzne	0.811	0.28	0.2	0.18
2	4,`4- dinitro azoxy benzene	1.15	0.65	0.4	0.3
3	4,`4- dichloro azoxy benzene	0.832	0.29	0.2	0.2
4	4-chloro-`4- methoxy azoxy benzene	0.521	0.49	0.3	0.19

With respect to the separation of the 4,`4-disubstituted azoxybenzenes studied, there is no methanol-water mobile phase which permits all of the azoxybenzenes to be sufficiently separated for quantitative determination(since I is not a liquid crystal, the separation and determination was discussed to be with the exclusion of I).

Consequently it is concluded that for any mixtures of the azoxybenzenes, qualitative and quantitative determination can be made with two methanol mobile phases containing an appropriate amount of water.The capacity factors of the azoxybenzenes studied were between five and sixteen with a methanol mobile phase containing 85% v/v of methanol.

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الخلاصة:

استخدمت تقنية كروموتوغرافيا السائل عالي الكفائة في فصل وتعيين مزيج من ازوكسي بنزين وثلاثة من مشتقاته هي 4،4´- ثنائي نايترو ازوكسي بنزين و 4،4 ثنائي كلورو ازوكسي بنزين و 4 كلورو4`-ميثوكسي ازاوكسي بنزين اذ ان دقة النتائج عالية جدا في هذه الطريقة لان نسب الخطأ كانت اقل من %(0.3) حيث ان الخطأ النسبي المئوي تراوح ما بين (0.28-0.65). واستخدام في هذه الطريقة طور متحرك عبارة عن مزيج من الكحول المثيلي والماء عند درجة حرارة ٥٠ ^{0}م وكانت نسبة الميثانول في هذا المزيج تتروواح ما بين % (80-90). وكان حدود الكشف في هذه الطريقة قد وصل الى ما يقارب من (0.2) نانوغرام.